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PLASMA CHOLECYSTOKININ AND HEPATIC ENZYMES, CHOLESTEROL AND LIPOPROTEINS IN AMMONIUM PERFLUOROOCTANOATE PRODUCTION WORKERS

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ABSTRACT

Ammonium perfluorooctanoate is a potent synthetic surfactant used in industrial applications. It rapidly dissociates in biologic media to perfluorooctanoate [$CF_3(CF_2)_6CO_2^-$], which is the anion of perfluorooctanoic acid [PFOA, $CF_3(CF_2)_6COOH$]. PFOA is a peroxisome proliferator known to increase the incidence of hepatic, pancreas and Leydig cell adenomas in rats. The pancreas acinar cell adenomas may be the consequence of a mild but sustained increase of cholecystokinin as a result of hepatic cholestasis. Although no significant clinical hepatic toxicity was observed, PFOA was reported to have modulated hepatic responses to obesity and alcohol consumption among production workers. To further assess these hypotheses, we examined medical surveillance data of male workers involved in ammonium perfluorooctanoate production in 1993 (n = 111), 1995 (n = 80) and 1997 (n = 74). Serum PFOA was measured by high-performance liquid chromatography mass spectrometry methods. Plasma cholecystokinin was measured (only in 1997) by the use of direct radioimmunoassay. Serum biochemical tests included hepatic enzymes, cholesterol and lipoproteins. Serum

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PFOA levels, by year, were: 1993 (mean 5.0 ppm, SD 12.2, median 1.1 ppm, range 0.0–80.0 ppm); 1995 (mean 6.8 ppm, SD 16.0, median 1.2 ppm, range 0.0–114.1 ppm); and 1997 (mean 6.4 ppm, SD 14.3, median 1.3 ppm, range 0.1–81.3 ppm). Cholecystokinin values (mean 28.5 pg/ml, SD 17.1, median 22.7 pg/ml, range 8.8–86.7 pg/ml) approximated the assay's reference range (up to 80 pg/ml) for a 12 hour fast and were negatively, not positively, associated with employees' serum PFOA levels. Our findings continue to suggest there is no significant clinical hepatic toxicity associated with PFOA levels as measured in this workforce. Unlike a previously reported observation, PFOA did not appear to modulate hepatic responses to either obesity or alcohol consumption. Limitations of these findings include: 1) the cross-sectional design as only 17 subjects were common for the three surveillance years; 2) the voluntary participation that ranged between 50 and 70 percent; and 3) the few subjects with serum levels $\geq$10 ppm.

INTRODUCTION

Ammonium perfluorooctanoate [APFO; $CF_3(CF_2)_6CO_2^-NH4^+$] is a potent synthetic surfactant used in industrial applications which rapidly dissociates in biologic media to perfluorooctanoate [$CF_3(CF_2)_6CO_2^-$], which is the anion of perfluorooctanoic acid [PFOA, $CF_3(CF_2)_6COOH$]. In laboratory animals, PFOA and its salts are: 1) absorbed by ingestion, inhalation or dermal contact;[1–3] 2) distributed primarily in the liver and blood;[4] 3) not biotransformed, conjugated, incorporated into lipids or form coenzyme A conjugates;[5–8] and 4) eliminated in the female rat at a greater rate of renal excretion than the male rat although no gender differences in excretion of PFOA have been seen in other laboratory animal species.[1,4,9] In rats, administration of APFO results in peroxisome proliferation, uncoupling of mitochondrial oxidative phosphorylation and altered lipid metabolism.[9,10,11] In a 90-day gavage study of rhesus monkeys, all animals in the 100 mg/kg/day group and 3 of the 4 animals in the 30 mg/kg/day group died before the end of study.[1,12] Histopathologic examination revealed marked diffuse lipid depletion in the adrenals, slight to moderate hypocellularity of bone marrow, moderate atrophy of lymphoid follicles in the spleen and moderate atrophy of the lymphoid follicles of the lymph nodes in the two highest treatment groups. There were no histopathologic changes in the 0, 3 and 10 mg/kg/day dose groups. In lifetime feeding bioassays of rats,[13,14] APFO in the diet at 300 ppm (daily dose of 15 mg/kg/day) increased the incidence of liver, Leydig cell and pancreas acinar cell adenomas. The liver and testicular tumors most likely occur via nongenotoxic mechanisms: oxidative stress and apoptosis in the development of the liver tumors; and enhanced hepatic aromatase activity which results in a hormone-mediated mechanism (increased estradiol) for the formation of Leydig cell tumors.[15–17] The pancreas acinar cell adenomas

were hypothesized to be a result of a mild but sustained increase in cholecystokinin (CCK) levels secondary to hepatic cholestasis.[18] CCK has been shown in animal models to produce pancreatic hypertrophy, hyperplasia and neoplasia.[19–23]

Hepatic toxicity and hypolipidemia have not been observed in APFO production workers.[24,25] Gilliland and Mandel did report that PFOA may negatively modulate the effect alcohol has on high-density lipoprotein (HDL) levels and exacerbate the effect that obesity has on hepatic enzyme tests.[25] This workforce was not found to be at an increased mortality risk for liver cancer or liver disease.[26] However, there were four pancreatic cancer deaths compared to two expected deaths (Standardized Mortality Ratio 2.0, 95% Confidence Interval 0.5–5.0). One of these four pancreatic cancer deaths had worked in the building where APFO was produced.

The purpose of this analysis was to examine several additional years of medical surveillance data at this APFO production plant in order to determine: 1) whether CCK levels are positively associated with serum PFOA levels among production employees; 2) whether PFOA results in clinical hepatic toxicity; and 3) whether PFOA may modulate hepatic responses to obesity and alcohol.

METHODS

PFOA Production

APFO production at this 3M plant began in 1947. APFO, a white powder, is produced via a five-stage process: electrochemical fluorination; isolating and converting the chemical to a salt slurry; converting the slurry to a salt cake; drying the cake; and packaging. The greatest likelihood for exposure has occurred in the drying area.

Subject Selection and Data Collection

Voluntary medical surveillance examinations were offered to the fluorochemical production workers in 1993, 1995 and 1997. The total number of male subjects, by year, who participated in these three cross-sectional investigations were: 1993 ($n = 111$); 1995 ($n = 80$); and 1997 ($n = 74$). (There were too few female employees to include in the data analysis.) Eligible voluntary participation rates among these production workers ranged from approximately 50 (1997) to 70 (1993) percent. There were 68 subjects in common for 1993 and 1995; 21 subjects in common between 1995 and 1997 (lower number due to employee turnover and re-assignments); and 17 subjects in common for all three years. Surveillance activities included a self-administered questionnaire, measurement of

height, weight and pulmonary function, standard biochemical and urinalysis tests, serum PFOA determination and several male reproductive hormone assays. The hormone data were collected in 1993 and 1995 and the findings have been reported elsewhere.[27] Serum hepatic and lipoprotein-related biochemical tests included: alkaline phosphatase (IU/L); gamma glutamyl transferase (GGT, IU/L); aspartate aminotransferase (AST, IU/L); alanine aminotransferase (ALT, IU/L); total bilirubin (mg/dl); direct bilirubin (mg/dl); total cholesterol (mg/dl); low-density lipoproteins (LDL, mg/dl); high-density lipoproteins (HDL, mg/dl); and triglycerides (mg/dl). In 1997, employees' plasma CCK-33 (pg/ml) levels were determined. CCK exists in various forms and lengths, although sulfated CCK-33 (i.e., a 33 amino-acid arrangement) appears to be the predominant form. For purposes of brevity, we will refer to CCK-33 as CCK. Employees were required to have fasted for 12 hours prior to their venipuncture. Because CCK analyses were not a standard analysis of the company's fluorochemical medical surveillance program, a study protocol was reviewed and approved by the company's human subjects committee and a signed, informed consent was obtained from each participant.

Serum chemistries and hematology were evaluated at Allina Laboratories (Minneapolis, Minnesota). Plasma CCK was measured by direct radioimmunoassay by Inter Science Institute (Inglewood, California). Serum PFOA (i.e., perfluorooctanoate) was determined by thermospray (1993 and 1995) and electrospray (1997) high-performance liquid chromatography/mass spectrometry methods.[28,29]

Data Analysis

Simple and stratified analyses, analysis of variance (ANOVA), and multivariable regression techniques were used to evaluate linear and nonlinear associations between PFOA and the biochemical parameters with adjustment for potential confounding variables.[30] Various serum category levels were used for the stratified analysis with no significant differences observed based on cutpoints as high as $\geq$30 ppm. However, the number of employees at $\geq$30 ppm was 5 or fewer (based on year). For purposes of this report, employees were stratified into three PFOA categories (0 – <1 ppm; 1 – <10 ppm; and $\geq$10 ppm) in order to provide a greater number of employees in the highest ($\geq$10 ppm) category. For multivariable regression analyses, PFOA, age, body mass index (BMI), alcohol use, and cigarette use were examined as both categorical and continuous variables. Alcohol use was analyzed as less than 1 drink per day, $\geq$1 drink per day and non-response to this questionnaire item (almost all subjects reported between <1–3 drinks/day). Linear and nonlinear transformations of PFOA were used to test for associations. In particular, the multivariable models employed by Gilliland

and Mandel[25] were used to determine whether PFOA has a modulating effect on obesity or alcohol consumption in regards to hepatic serum chemistries (ALT, AST and GGT) and HDL, respectively.

RESULTS

Serum PFOA levels, by year, were: 1993 (mean 5.0 ppm, SD 12.2, median 1.1 ppm, range 0.0–80.0 ppm); 1995 (mean 6.8 ppm, SD 16.0, median 1.2 ppm, range 0.0–114.1 ppm); and 1997 (mean 6.4 ppm, SD 14.3, median 1.3 ppm, range 0.1–81.3 ppm). Provided in Table I are the mean, standard deviation, median and range of the employees' age, BMI and hepatic, cholesterol and lipoprotein serum chemistry data stratified by PFOA level and the year of the medical surveillance examination. Depending upon the surveillance year, one to two orders of magnitude of difference were observed between the means (and medians) of the lowest and highest serum PFOA categories. There was no evidence for abnormal liver function tests, hypolipidemia or cholestasis associated with increasing employees' serum PFOA levels. Controlling for potential confounders, multivariable regression analyses did not suggest otherwise. Other measures including renal function, blood glucose and hematology were not associated with serum PFOA levels (data not shown).

The mean CCK value was 50 percent lower among employees with serum PFOA values $\geq$10 ppm than for those employees with serum PFOA levels $<$1 ppm (Table I), Figure 1 is a scatter plot of the natural log of CCK and PFOA. All but two CCK values were within the assay's reference range (up to 80 pg/ml). These two CCK values (80.5 pg/ml and 86.7 pg/ml) were of employees with 0.6 ppm and 5.6 ppm serum PFOA levels, respectively. Adjusting for potential confounding variables, we continued to observe a negative association between the natural log of CCK and serum PFOA levels (Table II) although minimum variation was explained ($R^2 = .08$).

Provided in Table III are the multivariable regression models (as originally reported by Gilliland and Mandel with the 1990 medical surveillance data[25]) which examined the potential modulating effect of PFOA on the association between alcohol and HDL. The coefficients of determination (R^2, adjusted R^2) were not large for any model. Based on these models, Table IV shows the change in HDL levels associated with a 10 ppm increase in serum PFOA levels among light and moderate drinkers ($\geq$1 drink/day) compared to light drinkers ($<$1 drink/day). [Note: total serum organic fluorine measurements, rather than serum PFOA, were used in 1990.] Unlike the findings from the 1990 data, the effect of alcohol use on increasing HDL levels was not blunted by a 10 ppm increase in PFOA in any of the subsequent medical surveillance examination years.

Table I. Mean, Median, Standard Deviation (S.D.) of Mean and Range of Demographic, Hepatic, Cholesterol, and Lipoprotein Values by Serum PFOA Categories and Medical Surveillance Year (1993, 1995, 1997)

PFOA* Category (ppm)	1993				1995				1997			
	Mean	Median	S.D.	Range	Mean	Median	S.D.	Range	Mean	Median	S.D.	Range
						PFOA (ppm)						
0–<1	0.48	0.48	0.27	0.00–0.99	0.31	0.20	0.32	0.00–0.90	0.47	0.55	0.26	0.05–0.92
1–<10	3.38	2.50	2.17	1.03–8.92	3.03	2.40	1.84	1.10–8.20	3.13	2.30	2.12	1.05–7.66
≥10	30.88	19.50	25.12	11.90–80.00	30.06	25.50	26.58	10.3–114.1	32.13	22.52	24.84	10.50–81.35
	F Value = 68.3, p = .0001				F Value = 39.1, p = .0001				F Value = 48.7, p = .0001			
						Age						
0–<1	43	41	9.2	27–61	42	41	8.3	29–60	40	39	9.1	25–61
1–<10	39	38	7.8	27–60	41	40	8.6	24–58	41	41	8.7	26–58
≥10	39	38	6.6	25–49	43	45	8.4	27–55	42	46	10.9	28–57
	F Value = 3.7, p = .02				F Value = 0.2, p = .85				F Value = 0.2, p = 0.81			
						BMI (kg/m^2)						
0–<1	28.0	27.6	4.3	20.9–42.0	27.6	26.8	4.2	21.9–45.2	28.7	27.5	3.9	21.5–35.0
1–<10	26.9	26.3	2.5	21.6–32.5	28.6	27.9	3.4	22.1–38.3	29.5	29.5	5.3	21.9–46.8
≥10	28.4	28.5	2.4	22.4–32.0	28.4	28.8	3.5	21.2–34.8	27.6	28.5	3.5	22.0–33.0
	F Value = 1.7, p = .14				F Value = 0.7, p = .51				F Value = 0.8, p = .47			
						CCK (pg/ml)						
0–<1	Not done in 1993				Not done in 1995				33.4	31.3	15.7	13.4–80.5
1–<10									28.0	21.1	19.2	8.8–86.7
≥10									17.4	16.4	5.6	11.4–29.9
									F Value = 3.8, p = 0.03			
						Alkaline Phosphatase (IU/L)						
0–<1	88	82	26	37–161	78	76	18	40–114	79	78	19	27–122
1–<10	82	78	23	47–151	80	76	25	48–165	87	84	23	47–164
≥10	83	75	24	58–132	89	76	31	55–146	80	73	23	61–142
	F Value = 0.7, p = .52				F Value = 1.4, p = .25				F Value = 1.3, p = .28			

						GGT (IU/L)						
0–<1	33	33	19	11–84	42	34	27	16–149	34	25	27	15–230
1–<10	50	48	70	6–472	51	36	41	19–190	36	32	25	14–162
≥10	37	37	17	19–77	40	38	13	21–61	30	29	10	15–50
		F Value = 1.5, p = .24				F Value = 0.9, p = .41				F Value = 0.2, p = .78		
						AST (IU/L)						
0–<1	23	24	7	11–60	21	20	6	13–36	26	23	7	13–41
1–<10	26	24	11	12–83	24	20	13	13–75	25	25	7	14–48
≥10	24	24	5	16–35	21	20	4	15–29	25	25	4	22–34
		F Value = 1.1, p = .33				F Value = 0.8, p = .45				F Value = 0.2, p = .83		
						ALT (IU/L)						
0–<1	45	45	14	22–88	44	40	13	27–80	31	30	10	13–59
1–<10	48	47	29	22–221	53	40	34	27–175	33	28	15	14–80
≥10	46	47	8	35–62	47	48	12	28–71	35	31	13	18–57
		F Value = 0.2, p = .82				F Value = 1.2, p = .30				F Value = 0.3, p = .73		
						Total Bilirubin (mg/dl)						
0–<1	0.6	0.6	0.3	0.2–1.3	0.8	0.8	0.3	0.4–2.2	0.8	0.7	0.5	0.4–2.3
1–<10	0.6	0.5	0.2	0.2–1.3	0.7	0.7	0.3	0.4–1.3	0.8	0.8	0.4	0.3–2.4
≥10	0.6	0.6	0.2	0.2–0.8	0.7	0.6	0.2	0.4–1.0	0.7	0.8	0.3	0.3–1.2
		F Value = 0.7, p = .48				F Value = 2.3, p = .11				F Value = 0.6, p = .58		
						Direct Bilirubin (mg/dl)						
0–<1	0.2	0.2	0.1	0.1–0.4	0.2	0.2	0.1	0.2–0.3	0.1	0.1	0.1	0.0–0.6
1–<10	0.2	0.2	0.1	0.1–0.3	0.2	0.2	0.1	0.1–0.3	0.1	0.1	0.1	0.0–0.2
≥10	0.2	0.2	0.1	0.1–0.3	0.2	0.2	0.1	0.1–0.3	0.1	0.1	0.1	0.0–0.1
		F Value = 0.2, p = .82				F Value = 3.2, p = .05				F Value = 0.3, p = .74		
						Cholesterol (mg/dl)						
0–<1	215	210	44	136–297	207	204	37	115–284	199	201	30	129–257
1–<10	219	213	39	155–309	212	210	36	143–284	213	213	41	121–315
≥10	232	227	37	171–305	221	220	41	132–288	229	217	46	164–334
		F Value = 0.8, p = .45				F Value = 0.8, p = .48				F Value = 2.7, p = .08		

(*continued*)

Table 1—*(continued)*

PFOA* Category (ppm)	1993 Mean	1993 Median	1993 S.D.	1993 Range	1995 Mean	1995 Median	1995 S.D.	1995 Range	1997 Mean	1997 Median	1997 S.D.	1997 Range
						HDL (mg/dl)						
0 - <1	43	42	11	22–83	42	41	8	22–59	41	40	9	26–61
1 - <10	47	48	13	28–90	43	45	12	22–67	44	44	12	23–94
≥10	48	44	21	27–97	41	42	9	26–54	45	46	10	24–60
	F Value = 1.2, p = .32				F Value = 0.4, p = .70				F Value = 0.9, p = .40			
						LDL (mg/dl)						
0 - <1	138	142	40	27–227	131	130	32	31–191	114	119	28	40–158
1 - <10	143	137	38	72–223	133	137	40	28–210	134	134	44	26–253
≥10	140	143	42	60–188	130	118	39	62–211	134	127	38	79–206
	F Value = 0.2, p = .84				F Value = 0.1, p = .96				F Value = 2.3, p = .11			
						Triglycerides (mg/dl)						
0 - <1	171	145	124	37–636	170	152	93	57–371	219	186	116	53–445
1 - <10	205	129	408	47–2845	175	123	144	59–743	176	161	85	44–360
≥10	221	223	159	41–564	254	183	154	77–563	251	188	192	63–718
	F Value = 0.3, p = .77				F Value = 2.7, p = .07				F Value = 2.1, p = .13			

*Study population size by PFOA category (ppm) and year.

Sample Size PFOA Category	1993	1995	1997
0 - <1	52	39	29
1 - <10	46	26	34
≥10	13	15	11
Total	111	80	74

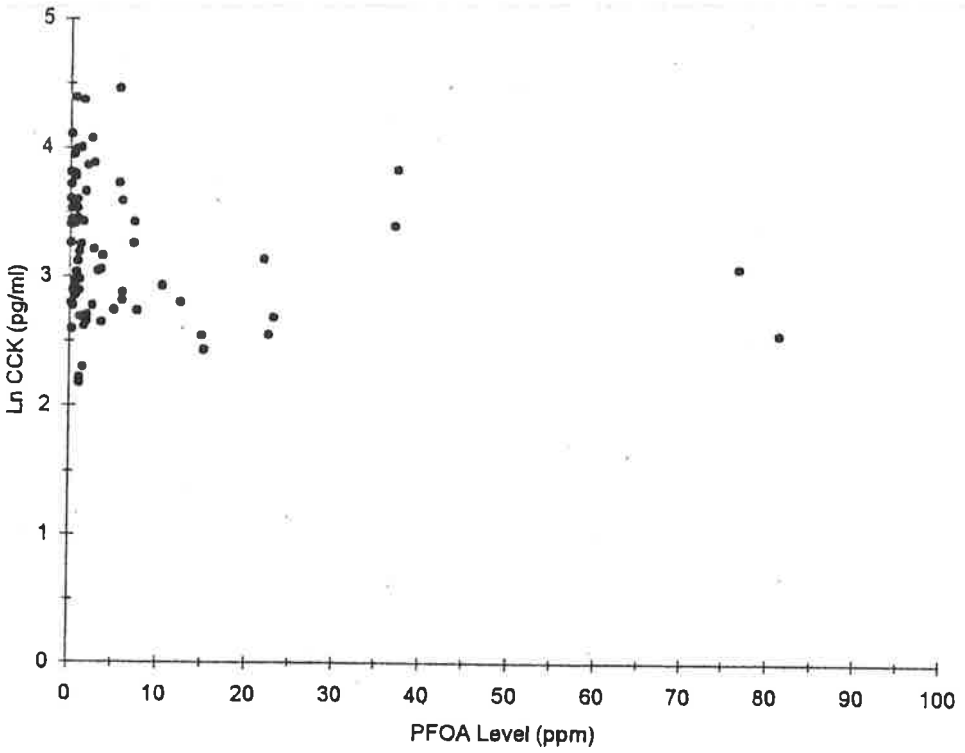

Figure 1. Scatter Plot of Natural Log CCK (pg/ml) by Serum PFOA Level (ppm) [Linear Regression Model: Ln CCK = 3.24–0.006PFOA; p value of PFOA coefficient = 0.19; $R^2 = .02$].

Table II. Multiple Regression Model of Factors Predicting the Natural Log of Plasma Cholecystokinin in Workers with Serum PFOA Levels

Variable	B	SE (B)	p value
Intercept	3.02	0.48	.0001
PFOA	−0.008	0.004	.07
Age	0.0001	0.007	.98
Alcohol	−0.005	0.086	.95
BMI	0.009	0.015	.53
Cigarettes	−0.009	0.007	.17

$R^2 = .08$
Adj $R^2 = .02$

Presented in Table V are multivariable regression models, including those originally reported using the 1990 surveillance data,[25] regarding the potential effect of PFOA on hepatic enzyme responses to obesity as measured by BMI. Again, the coefficients of determination were not large for any

Table III. Multivariable Regression Models* of Factors Predicting High Density Lipoprotein in Workers with Serum PFOA Levels

	1990			1993			1995			1997		
Variable	*B*	SE(*B*)	p value	*B*	SE(*B*)	p value	*B*	SE(*B*)	p value	*B*	SE(*B*)	p value
Intercept	65.00	10.07	.0001	55.00	16.70	.001	52.10	11.92	.0001	57.82	10.15	.0001
PFOA	−1.61	0.77	.04	−0.14	0.33	.67	−0.10	0.08	.18	−0.19	0.13	.16
Light Drink	−9.92	3.51	.006	−4.83	3.76	.20	−5.11	2.61	.05	−5.75	3.13	.07
Interaction#	1.62	0.80	.04	0.02	0.35	.96	0.02	0.13	.87	0.36	0.18	.05
	$R^2 = .17$			$R^2 = .10$			$R^2 = .30$			$R^2 = .11$		
	Adj R^2 = Not reported			Adj $R^2 = .02$			Adj $R^2 = .19$			Adj $R^2 = .03$		

*Adjusted for age, BMI, cigarette use, and non-respondents to alcohol question (all four years) and testosterone level (1990, 1993 and 1995). The 1990 regression model used total organic fluorine as the dependent variable (see reference #25).
#Interaction = PFOA × Light Drink

Table IV. Change in HDL* from Light Alcohol Drinker (<1 drink/day) to: 1) a Light Drinker with a 10 ppm Change in Serum PFOA; 2) a Moderate Alcohol Drinker (≥1 drink/day); and 3) a Moderate Alcohol Drinker with a 10 ppm Change in Serum PFOA

	Light Drinker with a 10 ppm increase in PFOA	Moderate Drinker	Moderate Drinker with a 10 ppm increase in PFOA
Year		Change in HDL (mg/dl)	
1990#	0.1	9.9	−6.2
1993	−1.3	4.8	3.4
1995	−0.8	5.1	4.1
1997	1.7	5.8	3.9

* Determined from multivariable models (see table 3) adjusted for age, body mass index, cigarette use, and non-respondents to alcohol question (all four years) and testosterone (1990, 1993 and 1995 only).
# Data in 1990 analyzed for total serum organic fluorine (see reference #25).

model. In the 1990 surveillance data, ALT increased among obese (BMI = 35 kg/m^2) but not non-obese (BMI = 25 kg/m^2) workers who had a 10 ppm change in serum PFOA (Table VI). However, this interaction was not observed in the 1993, 1995 or 1997 medical surveillance examinations. Likewise, we did not observe associations with AST or GGT (data not shown) as was reported in the 1990 medical surveillance examinations.[25]

DISCUSSION

We observed a negative association between serum PFOA and plasma CCK among 74 workers engaged in the production of APFO. This observation is opposite that proposed by Obourn et al who questioned whether chronic exposure to peroxisome proliferators, like PFOA, can cause pancreatic adenomas in the rat as the consequence of a mild but sustained increase in CCK levels secondary to hepatic cholestasis.[17] We do not believe the negative association observed in our study represents an entirely different biological relationship than what was originally postulated because: 1) all CCK values observed in this study were within the assay's reference range except for two values (which were not associated with high serum PFOA values); and 2) there was no suggestion of cholestasis which was considered the underlying reason for the elevated CCK levels in the rat.

There are several explanations for the lack of a positive association between PFOA and CCK in our study. First and foremost, the serum PFOA measurements in these production workers may have been too low to cause an increase in CCK if such a mechanism exists in humans. Second, the mechanistic reason for the elevated CCK levels in the Obourn et al study[17] was not clearly established. Obourn et al examined the effects of

Table V. Multivariable Regression Models* of Factors Predicting ALT in Workers with Serum PFOA Levels

	1990			1993			1995			1997		
	B	SE(*B*)	p value	*B*	SE(*B*)	p value	*B*	SE(*B*)	p value	*B*	SE(*B*)	p value
Intercept	58.13	24.60	.02	27.32	19.13	.16	40.93	22.90	.08	3.93	11.34	.73
PFOA	−15.80	4.58	.0008	0.89	2.88	.76	0.81	2.62	.75	2.77	1.27	.03
BMI	0.30	0.82	.72	1.07	0.67	.11	1.08	0.74	.15	1.54	0.35	.0001
Interaction#	0.62	0.17	.0004	−0.03	0.10	.79	−0.03	0.09	.76	−0.09	0.42	.04
	$R^2 = .21$			$R^2 = .06$			$R^2 = .11$			$R^2 = .32$		
	Adj R^2 = Not reported			Adj $R^2 = .01$			Adj $R^2 = .01$			Adj $R^2 = .26$		

*Adjusted for age, alcohol and cigarette use. The 1990 regression model used total organic fluorine as the dependent variable (see reference #25).
#Interaction = PFOA × BMI

Table VI. Change in Alanine Aminotransferase (ALT)* Associated with a 10 ppm Change in Serum PFOA for Three Body Mass Indices

	BMI (kg/m^2)		
	25	30	35
Year	Change in ALT (IU/L)		
1990[#]	−3.0	28.0	59.0
1993	2.2	0.9	−0.5
1995	1.5	0.1	−1.2
1997	5.3	0.8	−3.7

* Determined from multivariable regression model (see table 5) adjusted for age, alcohol and cigarette use.
[#] Data in 1990 analyzed total serum organic fluorine (see reference #26).

Wyeth 14,643, a more potent peroxisome proliferator than PFOA; thus their findings may not be directly related to PFOA. The clinical pathology data indicative of cholestasis included alterations in bile flow and bile acid output. Absolute bile flow and flow relative-to-body weight were marginally increased and acinar cell proliferation, although numerically increased at 3 months, returned to control levels at 6 months. Obourn et al also conducted *in vitro* experiments of both Wyeth-14,643 and PFOA which argued against other biological pathways known to elevate plasma CCK levels including CCK-A receptor agonism, trypsin inhibition and increased dietary fat content. Third, the primary set of biochemical and cellular events identified in rodents susceptible to the hepatic tumor effects of peroxisome proliferators has not been identified in either liver biopsies from humans exposed to peroxisome proliferators or in *in vitro* studies with human hepatocytes; however, the peroxisome proliferator-activated receptor (PPAR-α) is expressed at very low levels in the human liver.[17,31] It should also be noted that unlike the pancreas of the rat, the human pancreas has no detectable CCK-A receptors and little to no mRNA for the receptor.[31–34] Finally, whether CCK initiates or promotes pancreatic cancer is a controversial issue.[35] Data from more than seventy laboratory animal studies have variably suggested that CCK has positive trophic effects, inhibitory effects, or no involvement in pancreatic tumor growth.[36] CCK has promoted growth of human pancreatic cancers in cell cultures.[37] On the other hand, fasting plasma concentrations of CCK in unresected pancreatic cancer patients did not differ from healthy controls.[38] Also acinar cell malignancies in rats are rare in the human.[39] Activation of the c-K-ras gene is frequent in both human and hamster pancreatic cancer but is not found in azaserine-induced pancreatic cancer acinar cell adenoma models in the rat.[40,41]

Neither Gilliland and Mandel[25] nor ourselves observed significant clinical hepatic toxicity associated with the serum PFOA levels measured in this workforce. In the three surveillance years we examined (1993, 1995 and 1997), 88 percent, 81 percent and 85 percent of those employees who volunteered had serum PFOA levels less than 10 ppm, respectively. In a laboratory study, no significant hepatic toxicity was reported for the lowest dose (3 mg/kg/day) group of rhesus monkeys administered APFO by gavage for 90 days.[1,12] One of four primates in the next highest dose (10 mg/kg/day group) developed anorexia and black stools during the course of the study. There were no other abnormalities reported for this group. Only one animal survived in the 30 mg/kg/day group and none survived in the highest (100 mg/kg/day) dose group. For the 3 and 10 mg/kg/day dose groups, their mean serum total organic fluorine levels were 54 and 67 ppm, respectively.[1,12] Sixty-nine percent of the molecular weight of PFOA is organic fluorine; therefore these total organic fluorine levels in the lower dose groups may correspond to serum PFOA levels of 80 to 100 ppm. Total organic fluorine levels were analyzed in the liver for two animals in each of the two lowest two dose groups and the means were 5 and 10 ppm, respectively.[12] Liver total organic fluorine levels were also analyzed for the 30 mg/kg/day ($n = 4$, mean = 98 ppm) and the 100 mg/kg/day ($n = 2$, mean = 213 ppm) dose groups; however, only the sole surviving animal in the 30 mg/kg/day group was analyzed for serum total organic fluorine (145 ppm which approximates 210 ppm of PFOA). The 30 mg/kg/day group did have higher hepatic tranaminase values after 30 days of compound administration than the control group. Serum chemistries were not performed for the 100 mg/kg/day group after the onset of compound administration. Relative (% body weight) liver weights were higher among the 30 mg/kg/day (3.84%) and 100 mg/kg/day (3.31%) treatment groups than the control (2.36%), 3 mg/kg/day (2.36%) and 10 mg/kg/day (2.32%) dose groups. No abnormal liver function results were observed among employees with the highest serum PFOA levels; nevertheless these workers have been restricted from potential high exposure workplace areas.

We were unable to replicate, in three subsequent medical surveillance examinations, an earlier investigation's finding that PFOA may modulate hepatic responses to obesity and alcohol.[25] Total serum organic fluorine was used as a surrogate variable for PFOA in the 1990 medical surveillance exams. The use of total serum organic fluorine constitutes additional potential exposure to perfluorocarbons; however, data suggest that PFOA would represent the greatest fraction of total serum organic fluorine levels in this employee population.[24] Another explanation for the disparate findings, in particular as related to BMI, is that there may have been measurement error regarding BMI in the original study as well as in our investigation. We have previously noted the lack of an expected positive association between BMI and estradiol in the 1990 data.[27] Yet in the present study we

did not observe the anticipated strong positive correlation between BMI and ALT except in 1997. Correlation coefficients (p value in parentheses) were: 1993, r = .16, (p = .09); 1995, r = .13, (p = .27); and 1997, r = .43 (p = .0001). Self-reported alcohol data collected in the occupational setting can be questioned for its reliability as well as validity. The latter was not feasible to address; however, to partially examine the issue of reliability, we examined the analyses of the 68 employees who participated both in 1993 and 1995. The data showed good correlation between these two years for the potential confounding factors of BMI (r = .94, p = .0001), alcohol consumption (r = .67, p = .0001) and cigarette smoking (r = .84, p = .0001).

Several additional issues are worthy of consideration. The medical surveillance program is voluntary. Overall participation rates declined from approximately 70 percent in 1993 to 50 percent in 1997. Serum PFOA levels could differ between participants and nonparticipants. The high turnover of employees between 1995 and 1997 detracted from the opportunity for a longitudinal assessment. In this regard, we did examine the change in several parameters among the 68 subjects in common for 1993 and 1995. For example, the average difference in serum PFOA was +0.1 ppm (Wilcoxin signed-rank test = −540.5, p = .0001), the mean change in ALT was +0.5 IU/L (Wilcoxin signed-rank test = 50.0, p = 0.6) and the mean change in cholesterol was −1.6 mg/dL (Wilcoxin signed-rank test = −60.0, p = 0.4). The change in serum PFOA levels did not predict, via regression analyses, the change in ALT or cholesterol. Besides the few employees in common across all three years of medical surveillance data (n = 17), another difference that occurred in 1997 was the method of analysis of PFOA changed from thermospray (1993 and 1995) to electrospray (1997) high-performance liquid chromatography mass spectrometry. Also, the laboratory reference range substantially changed for ALT in 1997 (as can be seen in Table 1 by examining the lower mean values for ALT in 1997). Finally, the issue remains that the lack of a clinical hepatotoxic effect reported by Gilliland and Mandel[25] and ourselves does not rule out the possibility that PFOA may result in a subclinical hepatic effect in this production population that we have yet to observe. Results from ongoing laboratory animal studies, including a 6 month APFO gelatin capsule feeding study of cynomolgus primates, may provide further insight into the direction of medical surveillance activities for this workforce.

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An Epidemiologic Investigation of Reproductive Hormones in Men with Occupational Exposure to Perfluorooctanoic Acid

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Perfluorooctanoic acid (PFOA), a potent synthetic surfactant used in industrial applications, is a peroxisome proliferator that has resulted in dose-related increases in hepatic, pancreatic acinar, and Leydig cell adenomas in laboratory animals. In addition, PFOA increased serum estradiol levels through the induction of hepatic aromatase activity. In 1993 and 1995, we conducted two cross-sectional studies of 111 and 80 production workers, respectively, and specifically measured their serum PFOA in relation to several reproductive hormones to determine whether such an effect occurs in humans. PFOA was not significantly associated with estradiol or testosterone in either year's study. A 10% increase in mean estradiol levels was observed among employees who had the highest levels of serum PFOA, although this association was confounded by body mass index. Neither was PFOA consistently associated with the other measured hormones. Our results provide reasonable assurance that, in this production setting, there were no significant hormonal changes associated with PFOA at the serum levels measured. Limitations of this investigation include its cross-sectional design, the few subjects exposed at the highest levels, and the lower levels of serum PFOA measured, compared with those levels reported to cause effects in laboratory animal studies.

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Although fluoride (inorganic ionic fluoride) was identified in human blood 140 years ago,[1,2] the presence of fluorine in a covalently bound organic state was first reported in 1968.[3–4] Guy subsequently identified perfluorooctanoic acid (PFOA, $C_7F_{15}CO_2H$) as a major component of the serum organic fluorine fraction.[5] Ammonium perfluorooctanoate, a potent synthetic surfactant used in industrial applications, rapidly dissociates in aqueous solution to PFOA.

In laboratory animals, PFOA acid, or its salts, is absorbed by ingestion, inhalation, or dermal exposure[6–8] and is not metabolized.[9,12] PFOA is distributed primarily in the plasma and liver of male rats and the liver, plasma, and kidney in female rats.[11] The major route of elimination is via urine and feces. In the female rat, there is a tenfold–greater renal excretion rate.[11,13,14] Castrated male rats treated with estradiol have PFOA urinary excretion rates similar to those of female rats.[10,11]

Peroxisome proliferators, like PFOA, are a diverse class of chemicals that cause hepatic peroxisome proliferation and enzyme induction, liver hyperplasia, and, in some instances, hepatocarcinogenesis in rats and mice.[15–21] Two-year feeding studies in Crl:CD BR (CD) rats at a maximum amount of 300 parts per million (ppm) PFOA showed liver adenomas and an increased incidence of pancreas acinar cell adeno-

mas[22,23] and Leydig cell adenomas.[17,22] PFOA is not mutagenic[6] and thus the induction of these tumors most likely occurs through nongenotoxic mechanisms such as oxidative stress.[19,24]

To determine whether the Leydig cell adenomas were the result of an endocrine-related mechanism, Cook et al[18] gavaged CD rats for 14 days with up to 50 mg/kg of ammonium perfluorooctanoate. A significant increase in serum estradiol and decrease in testosterone levels were observed. The estradiol increase may be due to an induction of hepatic aromatase activity.[18] The decrease in serum testosterone levels might be the result of reduced conversion of 17-alpha hydroxyprogesterone (17-HP) to androstenedione (via the inhibition of the C-17,20 lyase enzyme). However, Biegel et al[19] were unable to replicate the negative testosterone association.

CD rats fed 100 ppm PFOA for a maximum of 13 weeks showed increased estradiol but not testosterone levels.[25,26] Elevated estradiol levels were found among CD rats fed 300 ppm during a 2-year bioassay, with no dose-related differences for testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH).[22]

The 3M Company has conducted medical surveillance of employees involved in PFOA production. Levels of serum organic fluorine (1.00–71.00 ppm) in production employees were 10- to 50-fold greater than values (0.01–0.13 ppm) reported from human sera.[27] Since PFOA could be an endocrine modulator,[18] a cross-sectional study among workers with potential exposure to PFOA was conducted in 1990.[28] Among 115 men engaged in PFOA production, total serum fluorine levels (sodium biphenyl extraction method[29]) ranged from 0 to 26 ppm (mean = 3.27 ppm; standard deviation [SD] = 4.68 ppm). It had been estimated that 80%–90% of human total serum fluorine levels consisted of PFOA.[27] Adjusting for potential covariates and hormones of a priori interest resulted in a positive nonlinear (quadratic) association with estradiol, positive linear associations with prolactin and TSH, and negative nonlinear (square root) associations with free or bound testosterone.[28]

A mortality study of employees in the chemical division, which included the PFOA production buildings, found no significantly increased cause-specific standardized mortality ratio for either male or female employees.[30] There were four deaths from prostate cancer, compared with 1.97 expected (95% confidence interval [CI], 0.55–4.59). Only one employee had worked directly in the PFOA production buildings. An association between PFOA exposure and prostate cancer was considered biologically plausible based on the animal and human data, which showed associations between PFOA and reproductive hormones.

The purpose of this report is to describe the results from two cross-sectional studies from the same plant that were done in 1993 and 1995.

Methods

PFOA Production

PFOA production at this plant began in 1947. PFOA, a white powder, is produced by an electrochemical process.[31] The products of this electrolysis cell reaction are highly fluorinated compounds, with the end-product defined by the starting material. Production involves a four-stage process: isolating and converting the chemical to a salt slurry, converting the slurry to a salt cake, drying the cake, and packaging. The greatest likelihood for exposure to PFOA occurred in the drying area.

Subject Selection

General medical surveillance is performed biennially for employees at this plant. There were 111 male employees in 1993 and 80 male employees in 1995 who participated in medical surveillance, hormone testing, and serum PFOA determination. Sixty-eight employees participated in both years. The surveillance consisted of a medical questionnaire; measurement of height, weight, and pulmonary function; standard biochemical and urinalysis tests; PFOA determination; and several hormone assays.

PFOA Analysis

A thermospray mass spectrophotometry assay was used to determine serum PFOA levels in 1993 and 1995.[32] The range of serum PFOA was 0 to 80 ppm in 1993 and 0 to 115 ppm in 1995. The upper limit of detection in 1993 was 80 ppm, whereas there was no upper limit of detection in 1995. Levels were highly correlated among the 68 employees studied in 1993 and 1995 (r = .91, P = 0.0001). There was also high correlation between total serum fluorine level measured with the 1990 study[28] and the PFOA measured in 1993 (r = .72, P = 0.0001, n = 94 subjects) and in 1995 (r = .84, P = 0.0001, n = 63 subjects). These findings were not unexpected, because of the estimated 18- to 24-month half-life of PFOA in humans.[27]

Hormone Assays

Serum samples were analyzed by the University of Minnesota's Endocrinology Laboratory (Minneapolis, MN) or the Endocrine Science Reference Laboratory (Tarzana, CA). Eleven hormones were assayed: cortisol, dehydroepiandrosterone sulfate (DHEAS), estradiol, FSH, 17α-hydroxyprogesterone (17-HP), free testosterone, total testosterone, LH, prolactin, thyroid-stimulating hormone (TSH) and sex hormone–binding globulin (SHBG). All but SHBG were analyzed at the University of Minnesota's Endocrinology Laboratory.

Cortisol was assayed using a fluorescence polarization immunoassay (Abbott TDx, North Chicago, IL). Radioimmunoassays (RIA) were used for DHEAS (Pantex, Santa Monica, CA), estradiol (modified

Pantex), 17-HP (modified CIS) and total testosterone (Coat-A Count; Diagnostic Product Corp., Los Angeles, CA). Free testosterone was determined using equilibrium dialysis. LH, FSH and prolactin were assayed using a microparticle enzyme immunoassay (Abbott Imx). TSH was determined using a chemiluminescence immunometric assay (Nichols, San Juan Capistrano, CA). SHBG was assessed via a radioimmunoassay after chromatographic sample purification (Endocrine Science Reference Laboratory). Bound testosterone was calculated as total testosterone less free testosterone. The same assays were used for both 1993 and 1995 analyses.

Data Analysis

Simple and stratified analyses, analysis of variance (ANOVA), Pearson correlation coefficients, and ordinary multivariable regression were used to evaluate associations between PFOA and each hormone, with adjustment for potential confounding variables. For stratified analyses, employees were divided into four PFOA categories: 0–1 ppm, 1-<10 ppm, 10-<30 ppm, and ≥30 ppm in order to determine if an effect existed at the highest serum levels. For multivariable evaluation, PFOA, age, body mass index (BMI), alcohol use, and cigarette use were examined as both categorical and continuous variables. Alcohol use was analyzed as less than 1 drink per day, 1–3 drinks per day, and non-response to the questionnaire item. Cigarette use was recorded as either current smoker or nonsmoker. Regression models were fitted with PFOA entered as a continuous variable, using linear, square, and square root transformations in order to assure that associations were not missed. The possible nonlinear association of estradiol, free testosterone, and bound testosterone was evaluated. Nonlinear dose-response relationships were examined by model fit and by comparing parameter estimates, using indicator and continuous variables. Stepwise selection procedures were also used. Study results were analyzed by the SAS System.[33]

We did not examine hormone changes between the two examinations because of the estimated half-life of PFOA (approximately two years) and intraindividual variability in hormones. Since the results for the 68 employees who participated in both years were similar to those obtained for the entire study, only the results for all employees are presented below.

Results

Serum PFOA levels were not highly correlated with either the covariates or the hormones. These correlation coefficients (in parentheses) for 1993 and 1995 data, respectively, for PFOA and the variable of interest were the following: age (−.22, .14); alcohol (.10, .18); BMI (.11, .10); cigarettes (.05, .11); cortisol (.07. −.05); DHEAS (.13, .12); estradiol (.12, .15); FSH (−.12, −.13); 17-HP (.11, .30); LH (−.06, .13); prolactin (.04, −.04); SHBG (−.07, .03); bound testosterone (.01, .02); free

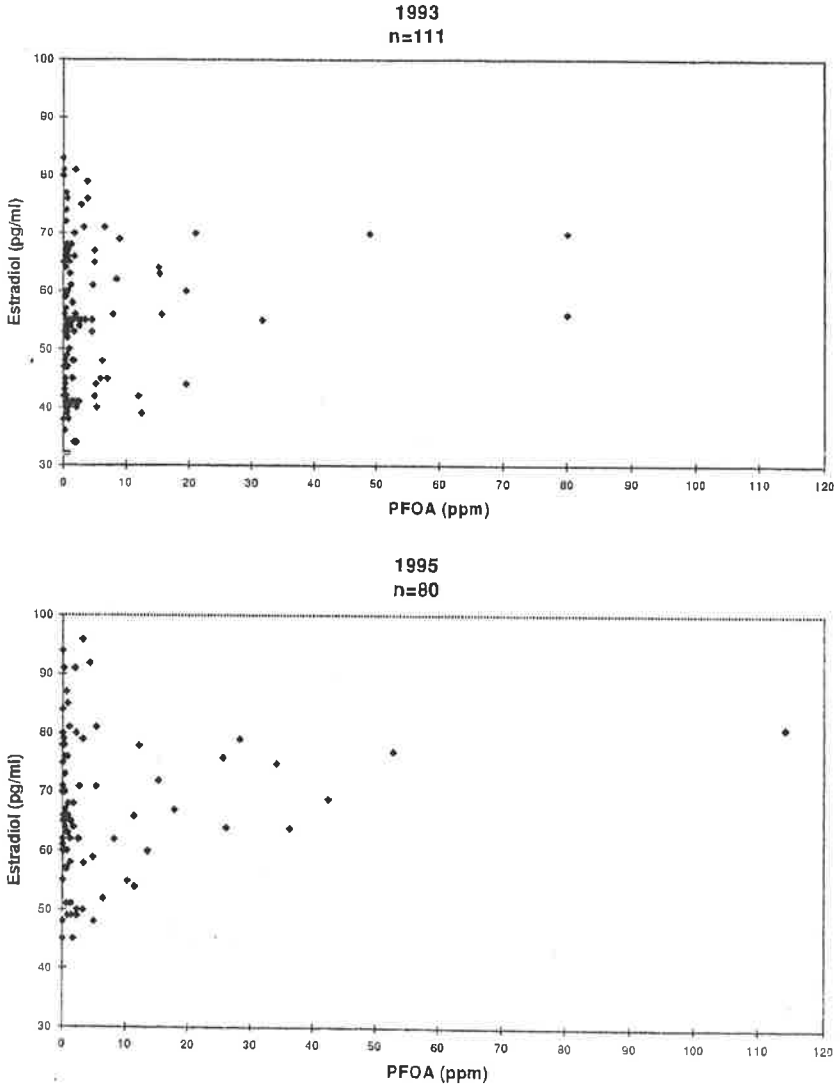

Fig. 1. Scatterplot of serum estradiol (pg/ml) by perfluorooctanoic acid (PFOA, in parts per million [ppm]) for employees in 1993 and 1995.

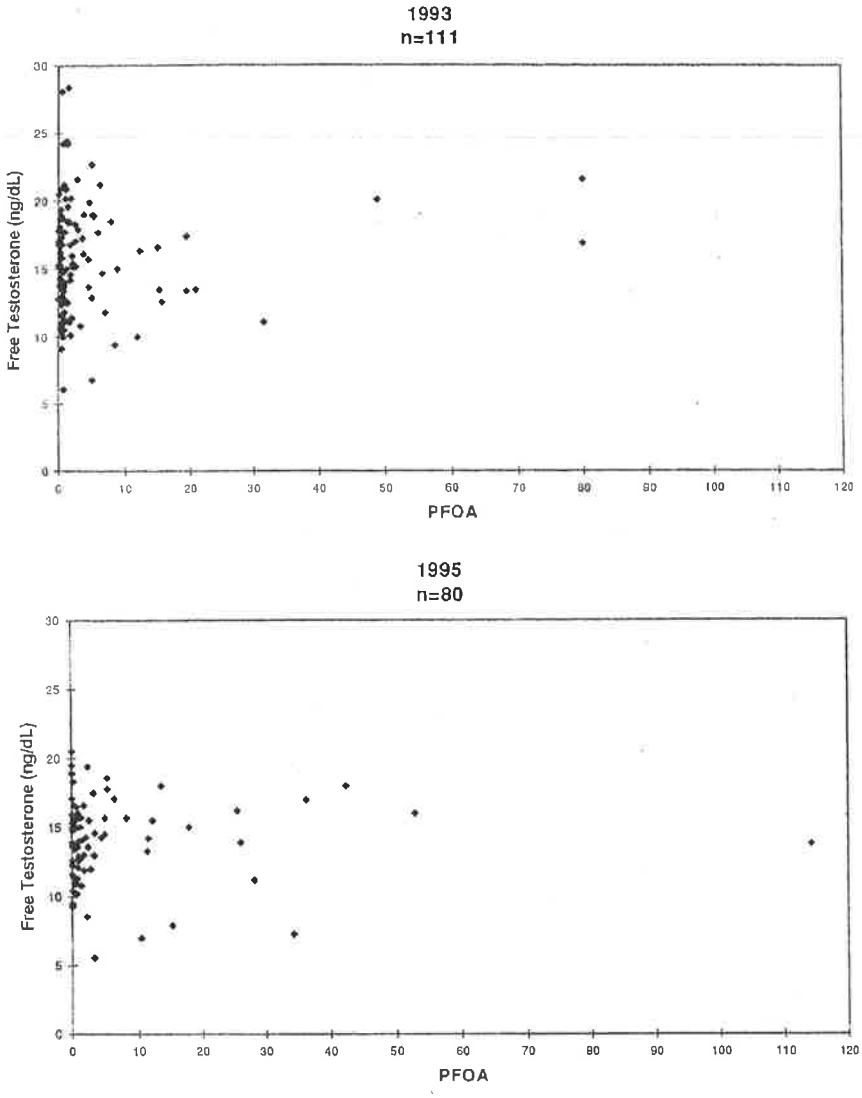

Fig. 2. Scatterplot of serum free testosterone (ng/dl) by PFOA (ppm) for employees in 1993 and 1995.

testosterone (.09, .01); and TSH (.03, .15).

Figures 1 and 2 are scatterplots for estradiol and free testosterone for each year. Simple linear regression of the natural log of each hormone with PFOA, treated as a continuous variable, resulted in no statistically significant coefficients in 1993 for any hormone and only one in 1995: 17-HP (beta coefficient = 0.006, $P = 0.03$, $R^2 = .06$). This result was dependent upon one person. In 1995, this person had a level of 198 ng/dL of 17-HP and 114 ppm of serum PFOA. In 1993, this person's values were 206 ng/dL of 17-HP and 80 ppm (upper limit of detection in 1993) for PFOA.

Table 1 provides the mean, median, standard deviation, and range of the covariates and several hormones, by four levels of PFOA categorization (0-<1, 1-<10, 10-< 30, and ≥30 ppm). Seventy-five percent of the employees with serum PFOA levels at 10 ppm or greater participated in both years. From Table 1, several observations are noteworthy. First, the mean of the PFOA ppm categories differed by two orders of magnitude between the lowest and highest categories for both years. Second, the ≥30-ppm PFOA category had the youngest mean employee age in both years. Third, BMI was the greatest among employees in the ≥30-ppm PFOA category in 1995. Fourth, mean estradiol levels were not significantly different between PFOA levels in either year, although the ≥30-ppm PFOA categories had mean estradiol levels that were 10% higher than the other PFOA levels. Fifth, there were no discernible trends between PFOA and either bound or free testosterone. Sixth, 17-HP levels were highest in the ≥30-ppm PFOA group in both years. No significant associations were observed for cortisol, DHEAS, FSH, LH, and SHBG (data not shown).

As expected,[34] estradiol was highly correlated with BMI (1993: $r = .41$, $P < 0.001$; 1995: $r = .30$, $P < 0.01$) and free testosterone with age (1993: $r = -.48$, $P < 0.001$; 1995: $r = -.40$, $P < 0.001$); thus Table 2 provides mean estradiol and free testosterone values stratified by BMI and age, respectively. It should be noted that all five employees in 1995 with serum PFOA levels ≥30 ppm had BMIs ≥28.

Linear and nonlinear relationships, taking into account potential confounders (especially age and BMI) as well as other covariates that may be on the biologic pathway of effect, resulted in no significant associations with PFOA except for 17-HP in the 1995 analyses (data not shown). Again, this association was dependent on the one employee discussed earlier.

Because a primary hypothesis of the present study was whether PFOA increased estradiol and decreased testosterone serum levels in a nonlinear fashion, we replicated these prior models[28] with our 1993 and 1995 data. PFOA was not significantly associated with serum estradiol, free testosterone, or bound testosterone (data not shown). There was no significant association (data not shown) between PFOA and prolactin among

TABLE 1
Mean, Median, Standard Deviation (SD) of Mean and Range of Perfluorooctanoic Acid (PFOA), Demographic and Hormonal Values by Serum PFOA Levels, and Year of Data Collection*

	1993 Data				1995 Data			
PFOA (ppm)	Mean	Median	SD	Range	Mean	Median	SD	Range
PFOA (ppm)								
0–<1†	0.48‡	0.47	0.27	0.00–0.99	0.31	0.2	0.32	0.00–0.90
1–<10	3.34‡	2.49	2.17	1.03–8.92	3.03	2.4	1.84	1.10–8.20
10–<30	16.26‡	15.40	3.39	11.90–21.00	17.11‡	14.3	6.90	10.30–28.20
≥30	60.13‡	64.45	24.01	31.60–80.00	55.96‡	42.4	33.29	34.20–114.10
	$F = 253.25$, $P = 0.0001$				$F = 77.57$, $P = 0.0001$			
Age (yr)								
0–<1	43.6	45.0	9.2	27.0–61.0	42.0	41.0	8.3	29.0–60.0
1–<10	39.2	38.0	7.7	27.0–60.0	41.3	40.0	8.6	24.0–58.0
10–<30	39.9	39.5	4.2	34.0–45.0	45.1	46.0	7.4	30.0–55.0
≥30	33.3	32.5	7.4	25.0–43.0	38.2	35.0	9.2	27.0–50.0
	$F = 3.67$, $P = 0.01$				$F = 0.88$, $P = 0.46$			
Alcohol (drinks/day)								
0–<1	0.4	0.3	0.5	0.0–1.9	0.5	0.3	0.7	0.0–2.9
1–<10	0.7	0.5	0.7	0.0–3.4	0.5	0.4	0.5	0.0–1.9
10–<30	0.9	0.7	0.6	0.4–2.1	0.8	0.7	0.7	0.0–2.1
≥30	0.9	0.7	0.8	0.0–2.0	0.5	0.4	0.6	0.0–1.4
	$F = 3.05$, $P = 0.03$				$F = 0.94$, $P = 0.43$			
BMI (kg/m^2)								
0–<1	28.0	27.5	4.2	20.9–42.0	27.6	26.8	4.2	21.9–45.2
1–<10	26.8	26.3	2.5	21.6–32.5	28.6	27.9	3.4	22.1–38.3
10–<30	29.1	28.8	1.8	27.1–32.0	27.8	27.7	4.0	21.2–34.8
≥30	28.5	28.4	1.6	26.9–30.2	29.8	28.9	1.8	28.2–32.6
	$F = 1.60$, $P = 0.19$				$F = 0.77$, $P = 0.52$			
Cigarettes (cigarettes/day)								
0–<1	2.6	0.0	7.5	0.0–30.0	3.8	0.0	9.4	0.0–40.0
1–<10	6.0	0.0	10.5	0.0–40.0	2.6	0.0	6.0	0.0–20.0
10–<30	2.5	0.0	7.1	0.0–20.0	9.1	0.0	15.2	0.0–40.0
≥30	5.0	0.0	10.0	0.0–20.0	6.0	0.0	8.9	0.0–20.0
	$F = 1.26$, $P = 0.29$				$F = 1.26$, $P = 0.30$			
Estradiol (pg/mL)								
0–<1	54.7	53.0	13.5	32.0–83.0	68.1	66.0	11.7	45.0–94.0
1–<10	56.0	55.0	12.0	34.0–81.0	65.2	62.0	14.9	45.0–96.0
10–<30	54.8	58.0	11.6	39.0–70.0	67.1	66.5	9.1	54.0–79.0
≥30	62.8	63.0	8.4	55.0–70.0	73.2	75.0	6.7	64.0–81.0
	$F = 0.54$, $P = 0.66$				$F = 0.69$, $P = 0.56$			
17-HP (ng/dL)								
0–<1	106.8	106.0	34.9	44.0–203.0	91.6	94.0	32.2	39.0–190.0
1–<10	120.2	115.5	41.5	45.0–249.0	110.6	105.5	35.6	54.0–179.0
10–<30	97.9	105.5	28.4	54.0–134.0	110.3	85.5	77.5	46.0–297.0
≥30	126.5	123.0	66.8	54.0–206.0	123.0	102.0	54.7	72.0–198.0
	$F = 1.55$, $P = 0.21$				$F = 1.67$, $P = 0.18$			
Prolactin (μg/L)								
0–<1	8.2	8.0	3.5	2.0–18.0	10.9	10.0	5.1	4.0–23.0
1–<10	8.8	8.0	4.6	2.0–22.0	11.8	10.0	6.0	5.0–28.0
10–<30	15.0§	9.0	15.2	6.0–51.0	12.9	14.0	5.3	3.0–21.0
≥30	7.5	7.5	0.6	7.0–8.0	9.4	9.0	2.7	7.0–14.0
	$F = 3.67$, $P = 0.01$				$F = 0.66$, $P = 0.58$			
Bound testosterone (ng/dL)								
0–<1	528.7	513.7	178.0	220.9–1059.5	534.8	518.5	150.4	278.7–1059.5
1–<10	609.7	609.2	168.2	212.2–1021.6	567.7	564.9	152.3	216.4–898.4
10–<30	485.2	477.5	113.9	301.0–651.6	554.4	549.7	185.5	238.1–823.8
≥30	569.6	596.5	81.6	450.9–634.4	567.8	623.0	155.0	341.7–703.0
	$F = 2.48$, $P = 0.07$				$F = 0.26$, $P = 0.85$			

TABLE 1
Continued

PFOA (ppm)	1993 Data Mean	Median	SD	Range	1995 Data Mean	Median	SD	Range
Free testosterone (ng/dL)								
0–<1	15.0	14.8	4.0	6.1–28.1	14.2	14.0	2.7	9.3–20.5
1–<10	16.6	16.5	4.4	6.8–28.4	14.2	14.4	3.1	5.6–19.4
10–<30	14.2	13.5	2.5	10.0–17.4	13.2	14.1	3.5	7.0–18.0
≥30	17.4	18.5	4.7	11.1–21.6	14.4	16.0	4.3	7.3–18.0
	$F = 1.81, P = 0.15$				$F = 0.31, P = 0.82$			
TSH (mU/L)								
0–<1	1.4	1.3	0.8	0.2–4.3	1.7	1.5	0.8	0.6–4.0
1–<10	1.4	1.2	0.7	0.5–3.1	1.7	1.5	0.9	0.5–3.7
10–<30	2.1	2.2	0.8	1.2–2.9	2.9§	2.5	1.1	1.9–5.8
≥30	1.2	1.1	0.4	0.8–1.8	1.7	1.3	0.6	1.1–2.5
	$F = 2.21, P = 0.09$				$F = 5.47, P = 0.002$			

* BMI, body mass index; 17-HP, 17-alpha hydroxyprogesterone; TSH, thyroid-stimulating hormone.
† Samples sizes: 0–<1 ppm: 1993, *n* 53; 1995, *n* = 39.
1–<10 ppm: 1993, *n* = 46; 1995, *n* = 26.
10–<30 ppm: 1993, *n* = 8; 1995, *n* = 10.
≥30 ppm: 1993, *n* = 4; 1995; *n* = 5.
‡ Mean significantly different (Bonferroni t-test, $p < .05$) than the three other PFOA ppm levels.
§ Mean level significantly different (Bonferroni t-test, $p < .05$) than the 0–<1 ppm and 1–<10 ppm PFOA categories.

TABLE 2
Mean, Median, Standard Error (SE) of Mean and Range of Estradiol by Body Mass Index and Free Testosterone by Age, Stratified by Serum PFOA Level and Year of Data Collection

BMI (kg/m²) by PFOA (ppm)	1993 Data *n*	Mean	Median	SE	Range	1995 Data *n*	Mean	Median	SE	Range
Estradiol (pg/mL)										
BMI <28										
0–<1 ppm	30	48.4	47.0	1.7	32.0–68.0	23	66.0	66.0	2.2	48.0–87.0
1–<10	30	55.0	55.0	2.2	34.0–81.0	13	62.0	62.0	3.7	48.0–91.0
10–<30	3	54.3	56.0	5.5	44.0–63.0	5	64.6	64.0	4.1	54.0–79.0
≥30	2	62.5	62.5	7.5	55.0–70.0	0	—	—	—	—
BMI ≥28										
0–1 ppm	23	63.0	66.0	2.9	32.0–83.0	16	71.1	72.0	3.2	45.0–94.0
1–<10	16	57.8	55.5	3.1	34.0–79.0	13	68.3	65.0	4.5	45.0–96.0
10–<30	5	55.0	60.0	6.1	39.0–70.0	5	69.6	72.0	4.1	55.0–78.0
≥30	2	63.0	63.0	7.0	56.0–70.0	5	73.2	75.0	3.0	64.0–81.0
Free testosterone (ng/dL)										
Age <40										
0–<1 ppm	20	17.3	16.8	0.9	10.5–28.1	18	15.3	15.2	0.6	11.3–20.5
1–<10	28	16.8	17.4	0.7	10.1–24.4	13	14.7	14.6	0.6	10.8–17.8
10–<30	4	15.2	14.9	1.0	13.4–17.4	2	15.7	15.7	2.4	13.3–18.0
≥30	3	19.5	20.1	1.4	16.9–21.6	3	15.9	16.0	1.2	13.8–18.0
Age ≥40										
0–<1 ppm	33	13.6	13.4	0.6	6.1–21.2	21	13.2	13.4	0.6	9.3–18.3
1–<10	18	16.2	15.8	1.3	6.8–28.4	13	13.7	14.3	1.0	5.6–19.4
10–<30	4	13.2	13.1	1.4	10.0–16.6	8	12.6	14.1	1.2	7.0–16.2
≥30	1	11.1	11.1	—	—	2	12.2	12.2	4.9	7.3–17.0

moderate drinkers, as was previously reported.[28]

Discussion

We conducted two cross-sectional studies of PFOA production workers to investigate the relation between serum PFOA levels and several reproductive hormones: in particular, estradiol and testosterone. Although we did not observe a significantly positive association between PFOA exposure and estradiol, mean estradiol levels were 10% greater among employees with the highest serum PFOA levels (≥30 ppm); however, this was confounded by BMI, and any interpretation is limited by the

few subjects at this PFOA level. Gilliland also observed an approximate 10% increase in mean estradiol levels from his lowest (0–1 ppm) to highest (15–26 ppm) total serum organic fluorine levels among these production employees.[28] Unlike the present study, this previous report also observed a significant nonlinear positive association between estradiol and total serum organic fluorine.[28] Possible reasons for the different results include the following: (1) use of different measurements of exposure (total serum organic fluorine in 1990 and serum PFOA in 1993 and 1995); (2) the possibility that the multivariate model used in 1990 may have transgressed the homoscedasticity assumption of regression analysis[35]; (3) possible misclassification of confounding variables (eg, the expected relationship between BMI and estradiol was not observed in 1990: correlation coefficient = −.01); (4) different subjects analyzed (94 employees participated in both the original 1990 and the 1993 surveys, compared with 61 employees who participated in 1990 and 1995); and (5) differences in the estradiol assays.

Dose, threshold effect, and species sensitivity may account for the apparent differences between the animal and human studies. We did not observe a significant association between estradiol and PFOA but did observe a 10% increase at the highest serum levels of PFOA. Serum PFOA in these workers was likely below the observable effect levels in animal studies; the observable effect level in the CD rat is somewhere above a mean serum level of 55 ppm PFOA.[18,25] All but three PFOA measurements from employees in our study were below 55 ppm PFOA. The 10% increase in mean estradiol levels observed among employees with the highest levels of serum PFOA (≥30 ppm) could suggest a threshold response. The discovery of the convergence of peroxisomal proliferators and estradiol at the level of their nuclear hormone receptors provides a plausible mechanism for a possible threshold relationship between PFOA and estradiol.[36,37] While responses to peroxisome proliferators, like PFOA, are readily observed in rats and mice, other species—including humans—have shown no such responses to many types of peroxisome proliferators at equivalent dose levels.[38–41]

We did not observe any significant associations between PFOA and free or bound testosterone. However, we did observe a significant positive association between 17-HP and PFOA in the 1995 but not 1993 analyses. We examined 17-HP, a precursor of testosterone, because Cook et al[18] suggested that PFOA may affect the conversion of 17-HP to testosterone via inhibition of 17,20-lyase. If this enzyme was inhibited, the expected result would be an increase in 17-HP levels, which was observed in both years' studies, although significantly in only the 1995 study. Recent laboratory work suggests that there may be an accommodation by the CD rat to the initial testosterone-lowering effect of PFOA.[19] A previous report[28] observed a significant negative nonlinear association between total serum fluorine and free or bound testosterone. This observation was dependent upon one influential data point, that of an employee who had no detectable total serum organic fluorine level but had the highest free testosterone level measured.

Several methodological issues should be considered in evaluating the results from this study. First, the cross-sectional design does not allow for a direct analysis of the temporality of an association. Given the long-half life of PFOA, it is conceivable that there may be some biological accommodation to the effects of PFOA, as suggested by Biegel et al.[19] Second, the two cross-sectional analyses cannot be viewed as independent populations because 68 employees were studied in both years. Fewer employees participated in serum measurements in the 1995 assessment, although the majority of those with the highest serum PFOA exposure levels in 1993 also participated in 1995. This reduced sample size resulted in lower statistical power. Third, we specifically measured serum PFOA levels. Use of total serum organic fluorine may represent other perfluorocarbons, which could be peroxisome proliferators, although data suggest that PFOA would represent the greatest fraction of total serum organic fluorine levels in this employee population.[27,29] Fourth, there could be measurement error in important confounding variables. Analysis of the 68 subjects who participated in both years showed good correlation for the confounding factors of BMI (r = .93, P = 0.0001) and the self-reported aspects of alcohol consumption (r = .67, P = 0.0001) and cigarette smoking (r = .84, P = 0.0001). Fifth, the quality of medical surveillance data can be evaluated by whether known associations are observed.[42] In this regard, we observed various expected associations (eg, estradiol and BMI, free testosterone and age). Finally, the pulsatile nature of some of the hormones studied (eg, FSH, LH, testosterone) has resulted in prior recommendations that mean hormone measurements should be the result of pooled blood from multiple samples taken at short intervals.[43] In our study, multiple samples were not feasible because of the low probability of employees voluntarily giving three samples over a 45- to 60-minute time period.

In summary, we conducted two cross-sectional studies in 1993 and 1995 and did not observe a significantly positive association between PFOA exposure and estradiol or a significantly negative association with testosterone. Our study may not have been sensitive enough to detect whether an association between PFOA and estradiol could exist in humans because measured serum PFOA levels were likely below the observable effect levels suggested in the animal studies. Our results provide reasonable assurance that sig-

nificant hormonal changes among these male production employees were not apparent in relation to their measured serum PFOA levels.

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Erratum

In the article by Olsen et al (Absenteeism among employees who participated in a workplace influenza immunization program. *J Occup Environ Med.* 1998;40:311–316), in the abstract and the last paragraph of the article, the word "heterogeneous" should have been "homogeneous."